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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: A. S. Greenberg Serial No.: 08/690,647 Filed: October 17, 2000 For: *Methods for Treating and Preventing Insulin Resistance and Related Disorders*	Examiner: Schmidt, M. Group Art Unit: 1635 Attorney Docket No.: TUV-005.01

Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration Under 37 C.F.R. § 1.132 by Andrew S. Greenberg

1. I, Andrew S. Greenberg, of Newton, Massachusetts, hereby declare as follows:

2. I am Director of the Program in Obesity & Metabolism, Energy Metabolism Laboratory, Jean Mayer USDA Human Nutrition, Research Center at Tufts University, Boston, Massachusetts, since 1993. I am also an Assistant Professor in Endocrinology and Molecular Medicine at Tufts Medical School, Boston, Massachusetts, since 1993 and an Assistant Professor at the School of Nutrition Science and Policy, Boston, Massachusetts, since 1995. I am a practicing doctor in the Division of Clinical Nutrition, Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine in the New England Medical Center, Boston, Massachusetts. Prior to holding these positions, I was a Medical and Senior Staff Fellow and then an Expert, Section of Membrane Regulation at the Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, Maryland since 1987. I have obtained my M.D. at New York University School of Medicine, New York, NY in 1981. My Curriculum Vitae including a list of my publications is attached as Exhibit I.

3. I am the sole inventor in the above-referenced patent application.

4. I have reviewed the present application (herein, the "Specification"), the pending claims, and the Office Action mailed on September 21, 2001 (herein, the "Office Action"). I understand that the Examiner has rejected the pending claims 1-14, *inter alia* on the grounds that "[t]here is no established correlation between administration of any possible inhibitor to cells in cell culture and administration to whole organisms as broadly claimed." It is also my understanding that the Examiner's comment applies in particular to the inhibitors that were pre-administered to the cells in culture.

5. A person of skill in the art would be without basis to reasonably doubt the asserted methods of treatment on its face. The specification describes that incubation of pre-adipocytes with an inhibitor of the MAPK pathway reduces the extent of lipolysis caused by TNF-α, relative to cells that were not incubated with an inhibitor of the MAPK pathway. This was demonstrated in the mouse 3T3-L1 cell line, as well as in primary cultures of human adipocytes (see, page 60, lines 19-23). Thus, based on the specification, a person of skill in the art would reasonably conclude that administration of an inhibitor of the MAPK pathway to a subject would similarly decrease the level of lipolysis in the subject and thereby treat any disease caused by or contributed to by lipolysis or elevated FFA levels.

6. To further substantiate any doubt that the results described in the patent application correlate reasonably with a use of inhibitors of the MAPK pathway for treating conditions caused by or contributed to by lipolysis or elevated FFA levels, I provide the following experimental data:

- I have confirmed that MAPK pathway inhibitors prevent basal and TNF-α induced lipolysis in fresh human cells even in the absence of pretreatment. I have prepared differentiated human adipocytes from fresh human tissue (surgical tissue). I also purchased similar fresh human cells from Zen-Bio (Research Triangle, NC). Both types of cells were incubated with PD98059 and with or without TNF-α for 6 and 24 hours and the level of glycerol was measured in the cultures. Similar results were obtained with both sources of adipocytes. The results indicated that PD98059 treatment decreased lipolysis in TNF-α treated cells to below control levels, and in non-TNF-α treated cells (PD98059 alone), to about 50% of control (Exhibit II). Thus, MAPK pathway inhibitors prevent basal and TNF-α induced lipolysis in fresh human cells even in the absence of pretreatment.
- I have obtained the same results with a different MAPK inhibitor. Here, I have used U0126, which, like PD98059, inhibits MEK, but is significantly more potent (Favata et al. (1998) *J. Biol. Chem.* 273:18623). Fresh human differentiated adipocytes were incubated for 24 hours with U0126 with or without TNF-α. The presence of U0126 decreased lipolysis in both TNF-α treated and non-TNF-α treated cells to 9.4% and 13.6% of control, respectively (Exhibit III), and reduced ERK1/2 phosphorylation to an undetectable level. Thus, the extent of inhibition of basal and TNF-α induced lipolysis correlates with the extent of ERK 1/2 inhibition, further substantiating that similar results would be obtained *in vivo*.

7. Thus, based on the above-results, it would be convincing to a person of skill in the art that a disease caused or contributed to by lipolysis or elevated FFA levels can be treated in a subject by administration to the subject of a MAPK pathway inhibitor.

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8 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Andrew S. Greenberg,

Dated: 2/21/02 Signature: [signature]